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ORIGINAL ARTICLE

Effect of excess levels of lysine and leucine in wheat-based, amino acid-fortified diets on the mRNA expression of two selected cationic amino acid transporters in pigs

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Summary

An experiment was conducted to evaluate the effect of excess levels of Leu and Lys on the expression of $b^{0,+}$ and CAT-1 mRNA in jejunum, liver and the muscles *Longissimus dorsi* (LDM) and *Semitendinosus* (STM). Twenty pigs with an average initial BW of 16.4 ± 1.7 kg were used in a Randomized Complete Block. Dietary treatments (T) were as follows: T1, basal diet; T2, basal plus 3.5 g L-Lys/kg diet; T3, basal plus 1.5 g L-Leu/kg diet; T4, basal plus 3.5 g L-Lys plus 1.5 g L-Leu/kg diet. Diets in T1 and T3 met 100% the requirement of Lys for pigs within the 10 to 20 kg body weight range; diets in T2 and T4 contained 35% excess of Lys. Also, diets in T1 and T2 supplied 104%, whereas diets in T3 and T4 supplied 116% the requirement of Leu. The expression of $b^{0,+}$ in jejunum was reduced ($p = 0.002$) because of the supplementation of L-Leu, but L-Lys supplementation had no effect ($p = 0.738$). In contrast, the expression of $b^{0,+}$ in STM ($p = 0.012$) and liver ($p = 0.095$) was reduced by the high level of Lys, but Leu had no effect ($p > 0.100$). CAT-1 expression in STM increased by high Lys ($p = 0.023$) and Leu ($p = 0.007$) levels. In liver, the expression of CAT-1 substantially increased ($p = 0.001$) because of Lys. In conclusion, excess levels of dietary Lys and Leu affect the expression of cationic amino acid transporters, and this effect varies depending on the studied tissue.

Introduction

The dietary amino acid (AA) content may affect their intestinal absorption and cell uptake in animals. Averous et al. (2003) indicated that a single AA deficiency results in the accumulation of its uncharged tRNA that activates the general control non-repressed protein 2 (GCN2) kinase. In turn, GCN2 kinase activates the translation of transcription factor 4 (GCN4; Hinnebusch, 1997) permitting translation initiation of vital proteins such as AA transporters (Kilberg et al., 2005). This indicates that a deficiency in one or more AAs stimulates the synthesis of their transporters. Studies conducted with yeast show that AA depletion activates translation of the cationic AA transporter (CAT-1) mRNA (Averous et al., 2003). However, there are no reports in mammals showing the effect of deficient, adequate or excessive levels of AAs in typical diets on the expression of AA transporters.

The transport of AA through the membrane cells of intestine, liver, and muscle is carried out by protein systems that show specificity for selected groups of AAs (Broer, 2008), being the cationic AAs systems (CAA-T) the most critical in pigs. Cationic AAs (Lys, Arg, His; CAAs) are transported at high affinity by

the Na-independent systems $b^{0,+}$ and y^+ (CAT-1; Majumder et al., 2009), which are the major CAA transporters in mammals (Palacin et al., 2001; Broer, 2008). The transporter $b^{0,+}$ is mainly expressed in epithelial cells, and CAT-1 is expressed in non-epithelial cells. The high affinity $b^{0,+}$ system exchanges Leu for Lys (Torras-Llort et al., 2001); the cellular uptake and intestinal absorption of CAAs by $b^{0,+}$ is coupled with the efflux of neutral AA, especially Leu (Pineda et al., 2004). This indicates that intracellular Leu stimulates the absorption of Lys. Thus, the abundance, affinity and activity of CAA-T proteins are expected to affect the intracellular availability of CAAs.

The abundance and activity of the CAA-T systems become crucial under regular feeding conditions because of the unbalanced AA profile in typical cereal-soybean meal diets for pigs (NRC, 1998). These diets are formulated to meet 100% the requirement of Lys, the first limiting AA, but contain excess of Arg and Leu (approximately 3× and 2× their requirement for growing pigs). Arg excess may compromise the availability of Lys for protein synthesis because they share the same transporter (Hagihira et al., 1961), but reducing the protein level coupled with the supplementation of free Lys could help to prevent this potential problem. On the other hand, Leu excess may increase the absorption of Lys, whereas the reduction in the protein content may prevent this effect. Although AA deficiencies stimulate the synthesis of AA transporters (Kilberg et al., 2005), there is no available information regarding the expression of CAA-T in pigs fed diets differing in AA content, especially in Lys and Leu. Understanding how the intestinal (enterocyte) and muscle (myocyte) cells regulate their capacity to absorb AAs is essential to manipulate diets to achieve maximal AA absorption (Liao et al., 2009) and efficient pig growth. Thus, an experiment was conducted to evaluate the effect of two levels (adequate and excess) of Lys and Leu in low protein diets containing sufficient contents of the remaining indispensable AA, on the expression of $b^{0,+}$ and CAT-1 mRNA in jejunum, liver and the muscles *Longissimus dorsi* (LDM) and *Semitendinosus* (STM).

Materials and methods

Animals

The experiment was conducted at the Metabolism and Physiology Unit of the Instituto de Ciencias Agrícolas, UABC. Twenty crossbred (Landrace × Hampshire × Duroc) pigs with an average initial BW of 16.4 ± 1.7 kg were used. Pigs were distributed in four groups based on initial body weight, age, sex and litter. Within each group, pigs were randomly assigned to one of four dietary treatments, according to a Randomized Complete Block design (Steel and Torrie, 1980); there were five replicates (three females and two males) per treatment. All pigs were individually housed in raised floor metabolism pens (1.2 m wide, 1.2 m long and 1.0 m high) equipped with a stainless-steel self-feeder attached to the front of the pen, a nipple water drinker next to the feeder, and iron mesh floor in a temperature-controlled room. Pigs were weighed at the beginning and the end of the 28-days trial. The average final BW of the pigs was 23.5 kg. The pigs used in these experiments were cared for in accordance with the guidelines established by the Canadian Council on Animal Care (1993).

Diets

A basal diet was formulated with wheat as the sole source of protein (Table 1) and was supplemented with 7.0 g L-Lys, 2.8 g L-Thr and 1.0 g DL-Met/kg, vitamins, minerals and corn starch. This diet contained sufficient levels of the entire indispensable AAs. Dietary treatments (T) were as follows: T1, basal diet; T2, basal plus additional 3.5 g L-Lys/kg; T3, basal plus 1.5 g L-Leu/kg; T4, basal plus additional 3.5 g L-Lys plus 1.5 g L-Leu/kg. Diets in T1 and T3 supplied 100% the requirement of Lys for pigs within the 10–20 kg body weight range (NRC, 1998), whereas in T2 and T4 diets contained 35%

Table 1 Formulation of the experimental diets (g/kg feed, as fed basis)

	Diet			
Ingredient	T1	T2	T3	T4
Wheat	957.2	957.2	957.2	957.2
L-Lys-HCl, 78%	9.0	13.5	9.0	13.5
L-Leu, 99%	–	–	1.5	1.5
L-Threonine, 99%	2.8	2.8	2.8	2.8
DL-Methionine, 99%	1.0	1.0	1.0	1.0
Corn starch	6.0	6.0	6.0	6.0
Calcium carbonate	13.5	13.5	13.5	13.5
Dicalcium phosphate	4.0	4.0	4.0	4.0
Iodized salt	3.5	3.5	3.5	3.5
Vitamin and mineral premix*	2.0	2.0	2.0	2.0

*Supplied per kilogram of diet: vitamin A, 4800 IU; vitamin D3, 800 IU; vitamin E, 4.8 IU; vitamin K3, 1.6 mg; riboflavin, 4 mg; D-pantothenic acid, 7.2 mg; niacin, 16 mg; vitamin B12, 12.8 μg; Zn, 64 mg; Fe, 64 mg; Cu, 4 mg; Mn, 4 mg; I, 0.36 mg and Se, 0.13 mg.

excess of Lys. On the other hand, diets in T1 and T2 supplied 104% the requirement of Leu, whereas in T3 and T4 diets supplied 116% the requirement (NRC, 1998). A single batch of wheat grown by irrigation was used. Crystalline L-Lys HCl (78%; Ajinomoto, Chicago, IL, USA) and L-Leu (Evonik Industries AG, Hanau, Germany) were supplemented to diets in T2–T4 at the expenses of corn starch. The amino acid composition of the experimental diets was calculated on the basis of the analysed amino acid composition of wheat, and crystalline Lys and Leu (Table 2). All diets were supplemented with sufficient amounts of Ca and available p and contained 10.04 MJ NE/kg.

Tissue collection

All pigs were slaughtered at the end of the 28-days trial by electrical stunning and exsanguination. The carcasses were immediately eviscerated, and samples (approximately 0.5 g) of mucosa scratched from middle jejunum were collected into 2-ml Eppendorf micro tubes; the proximal jejunum is the major site where the amino acid and peptide absorption occurs (Silk et al., 1985; Broer, 2008). Samples (0.5–1.0 g) from liver as well as the *Longissimus dorsi* and *Semitendinosus* muscles were collected and immediately stored in liquid nitrogen. The total collection process took 10 min or less. At the end of the sampling, all samples were transported to the molecular biology laboratory and stored at −82 °C until analysis.

RNA extraction and reverse transcription

Total RNA extraction and purification

The samples from jejunal mucosa, liver, LDM and STM were treated to extract total RNA by pulverization into liquid nitrogen and then following the instructions for the Trizol reagent (Invitrogen, Corp. Carlsbad, CA, USA). Purified RNA was then eluted with 30 μl of nuclease-free distilled water and stored at −82 °C. The concentration of total RNA was determined spectrophotometrically (Helios *β*; Thermo Electron, Rochester, NY, USA) at 260 nm, and purity of RNA was assessed by using the A260/A280 ratio, which ranged from 1.8 to 2.0 (Sambrook and Russel, 2001). The integrity of total RNA was evaluated by gel electrophoresis on 1% agarose gels. All RNA samples had good quality with a 28S:18S rRNA ratio around 2.0:1 (Sambrook and Russel, 2001).

Reverse transcription

Approximately 2 μg of total RNA were treated with 1 U of DNase I (1 U/μl; Invitrogen) and 6 μl of 5× reverse transcription buffer in a 30 μl reaction completed with diethylpyrocarbonate-treated water; the reaction was carried out during 15 min at room temperature and another 15 min at 70 °C. Reverse transcription was initiated with DNase-treated RNA samples, adding 1 μl of random primers (150 ng/μl) and 1 μl of dNTPs solution (10 μM each), and the reaction was incubated at room temperature and then chilled on ice for 1 min; 3 μl of dithiothreitol (0.1 M), 1 μl of RNase OUT (40 U/μl, Invitrogen) and 2 μl of 5× reverse transcription buffer were added to the reaction and incubated at 42 °C during 2 min to stabilize the reaction before adding 1 μl of RT-Superscript III reverse transcriptase enzyme (200 U/μl; Invitrogen). Reaction was incubated at 42 °C during 50 min. The mixture was incubated at 70 °C for 15 min and then chilled on ice to stop the reaction. cDNA samples were quantified spectrophotometrically and diluted into a final concentration of 50 ng/μl.

Table 2 Amino acid composition (g/kg, as fed basis) of the experimental diets; calculated on the basis of the analysed amino acid composition of wheat, and crystalline Lys and Leu

	Diet			
Indispensable amino acid	T1	T2	T3	T4
Arginine	6.6	6.6	6.6	6.6
Histidine	3.2	3.2	3.2	3.2
Isoleucine	5.1	5.1	5.1	5.1
Leucine	8.6	8.6	11.1	11.1
Lysine	10.8	14.3	10.8	14.3
Methionine	1.8	1.8	1.8	1.8
Phenylalanine	6.5	6.5	6.5	6.5
Threonine	6.5	6.5	6.5	6.5
Valine	6.2	6.2	6.2	6.2

Real-time PCR

Specific primers for each amino acid transporter mRNA were designed according to their published sequences at the Genbank (Table 3). Also, a housekeeping 18S rRNA gene (GenBank AY265350) was used as an endogenous control to normalize variations in mRNA. Before starting, end-point PCR was carried out to standardize the amplification conditions for each pair of primers, and to confirm the specificity of the PCR products related to its mRNA, a sample of every PCR products was cloned into a TOPO vector 4.0 (Invitrogen) and sequenced at the Davis Sequencing Facility (Davis, CA, USA).

Table 3 Primers used for the qPCR analyses of cDNA derived from cationic amino acid transporters mRNA and 18S ribosomal RNA

mRNA	Primer	Location (bp) on the template	Sequence	Amplicon size (bp)
CAT-1 (GenBank: AY371320.1)	Forward	4239–4258	5′GTCGGTTGCAAAGACCATTT3′	329
	Reverse	4548–4567	5′GAGCGGTGCTGACAACAGTA3′	
$b^{0,+}$AT (SLC7A9) (GenBank: EF127857.1)	Forward	1–19	5′CGGAGAGAGGATGAGAAGT3′	562
	Reverse	545–562	5′GCCCGCTGATGATGATGA3′	
18S rRNA (GenBank: AY265350.1)	Forward	236–255	5′GGCCTCACTAAACCATCCAA3′	295
	Reverse	511–530	5′TAGAGGGACAAGTGGCGTTC3′	

Expression of AA transporters in jejunum, muscles and liver was estimated by quantitative PCR (qPCR) assays, using the Maxima SYBR Green/ROX qPCR Master Mix (Fermentas, Corp. Glen Burnie, MD, USA) into a Chromo 4-DNA Engine, with the MJ Opticon Monitor 3.1 software (Bio-Rad, Herefordshire, England). The equipment was calibrated with a standard curve using the 18S rRNA cloned into a TOPO vector 4.0, from which a calibrator's cDNA was produced. The standard curve was obtained using known concentrations of 100-fold serial dilutions of the cDNA. Reactions for qPCR contained 50 ng of cDNA, 0.5 μM of each specific primer, 12.5 μl of 2× SYBR green/ROX qPCR Master Mix and DNase/RNase-free water to complete a final volume of 25 μl. 18S rRNA was used also as housekeeping gene to standardized the amount of amplified DNA. The PCR conditions used for the amplification and quantification were an initial denaturing stage (95 °C for 1 min), followed by 45 cycles of amplification (denaturing at 95 °C for 30 s; annealing at 56 °C for 15 s; and extension at 72 °C for 30 s) and a melting curve program (60–90 °C). Fluorescence was measured at the end of every cycle and every 0.2 °C during the melting program.

Statistical analysis

Analyses of variance of the data from each variable were performed using the GM of SAS (SAS, 2000). The effect of Lys, Leu and their interaction was tested; when the interaction was significant, contrasts were performed to test the effect of the level of one AA within one level of the other AA. Effects and differences were considered significant at $p > 0.10$.

Results

The real-time qPCR assays conducted in this study were validated by sequencing their final products. Sequencing results obtained at the Davis Sequencing Facility (Davis, CA, USA) revealed that the products for $b^{0,+}$, CAT-1 and 18S rRNA have 99% homology with their corresponding expected sequences acquired from the virtual template sequences reported in GenBank. Numerous studies have revealed that 18S rRNA expression is very stable and its content can be used as an endogenous control to normalize the expression of other genes in response to various stimuli (Liao et al., 2008). In this study, we also chose to normalize the relative expression of $b^{0,+}$ and CAT-1 mRNA to 18S rRNA expression. Accordingly, the 18S rRNA expression levels were used to normalize the relative quantities of each AA transporter-associated mRNA expression by jejunal mucosa, liver and muscles.

Expression of $b^{0,+}$ in jejunum, LDM, STM and liver

The results of the expression of $b^{0,+}$ in jejunum, LDM, STM and liver are presented in Table 4. Supplementation of L-Leu reduced the expression of $b^{0,+}$ in jejunum ($p = 0.002$), but Lys supplementation had no effect ($p = 0.738$). In LDM, the interaction Leu × Lys was significant ($p = 0.003$); thus, the effect of supplementing L-Lys at each level of Leu was tested. Supplementing L-Lys to the low Leu diet (T1 vs. T2) increased the expression of the transporter ($p < 0.001$). But, when L-Lys was supplemented to the high Leu diet, the expression of $b^{0,+}$ was not affected ($p > 0.100$). In contrast, supplementing L-Leu to the high Lys diet decreased the expression ($p = 0.002$), but in the low Lys diet, the expression was not affected ($p > 0.100$). In the STM ($p = 0.921$) and liver ($p = 0.328$), Leu did not affect the expression of $b^{0,+}$; but Lys reduced the expression in STM ($p = 0.012$) and liver ($p = 0.095$).

Expression of CAT-1 in jejunum, LDM and STM, and liver

The expression of CAT-1 in jejunum, LDM, STM and liver is presented in Table 5. There was no effect of

Table 4 Effect of supplementing L-leucine (Leu) and L-lysine (Lys) to wheat-based diets on the relative expression of the amino acid transporter protein $b^{0,+}$ in jejunum, the *Longissimus dorsi* (LDM) and the *Semitendinosus* (STM) muscles, and liver of growing pigs (arbitrary units; ratio of $b^{0,+}$ mRNA:18S rRNA)

Treatment	T1	T2	T3	T4				
	Supplemented amino acid, g/kg diet							
L-Leucine	0.0	0.0	1.5	1.5	Main effects, (p)			
L-Lysine	0.0	3.5	0.0	3.5	SEM	Leu	Lys	Leu × Lys
Jejunum	1.843	2.486	0.434	0.121	0.485	0.002	0.738	0.343
LDM*,†	1.236	3.713	1.892	1.505	0.384	0.066	0.018	0.003
STM	0.803	0.452	0.810	0.424	0.124	0.921	0.012	0.900
Liver	1.412	0.958	4.260	0.492	1.167	0.328	0.095	0.181

*C1: Effect of lysine, at the low leucine level (T1 vs. T2) $p < 0.001$.
†C1: Effect of leucine, at the high lysine level (T2 vs. T4) $p = 0.002$.

Table 5 Effect of supplementing L-leucine (Leu) and L-lysine (Lys) to wheat-based diets on the relative expression of the cationic amino acid transporter CAT-1 in jejunum, the *Longissimus dorsi* (LDM) and the *Semitendinosus* (STM) muscles, and liver of growing pigs (arbitrary units; ratio of CAT-1 mRNA:18S rRNA)

Treatment	T1	T2	T3	T4				
	Supplemented amino acid, g/kg diet							
L-Leucine	0.0	0.0	1.5	1.5	Main effects, (p)			
L-Lysine	0.0	3.5	0.0	3.5	SEM	Leu	Lys	Leu × Lys
Jejunum	0.100	0.179	0.129	0.210	0.056	0.601	0.178	0.982
LDM	0.691	1.377	0.682	1.076	0.304	0.621	0.100	0.639
STM	0.330	0.102	0.543	0.380	0.075	0.007	0.023	0.670
Liver	0.925	19.149	0.362	3.120	2.024	0.847	0.001	0.548

Leu on the expression of CAT-1 in jejunum ($p = 0.601$), LDM ($p = 0.621$) or liver ($p = 0.847$), but increased it in STM ($p = 0.007$). Lys did not affect either CAT-1 expression in jejunum ($p = 0.178$) or LDM ($p = 0.100$). However, Lys decreased the expression of CAT-1 in STM ($p = 0.023$) and increased it in liver ($p < 0.001$).

Relative expression of $b^{0,+}$ and CAT-1

The expression of mRNA for $b^{0,+}$ and CAT-1 in jejunum, LDM, STM and liver in growing pigs, in response to the individual or combined supplementation of L-Lys and L-Leu, relative to that in pigs fed the basal diet, is also presented (Table 6). The expression in pigs fed the basal diet was given an arbitrary value of 1; so, the values given to pigs fed the diets supplemented with L-Lys, or L-Leu, or both, represent the increase (if >1) or decrease (if <1) in the mRNA expression of each AA transporter relative to the basal diet. In jejunum, Leu alone decreased ($p = 0.063$) 76 percentage units (pu) the expression of $b^{0,+}$, and the combined supplementation of L-Lys and L-Leu reduced ($p = 0.028$) the expression by 93 pu. Expression of $b^{0,+}$ in LDM increased by 3.0× with the supplementation of L-Lys alone ($p = 0.001$). In STM, Lys alone ($p = 0.067$) or in combination with Leu ($p = 0.050$) decreased the expression of $b^{0,+}$. In liver, Lys or Leu alone or in combination did not change the expression of $b^{0,+}$. The expression of CAT-1 in jejunum and LDM was not affected by the individual or combined supplementation of L-Lys and L-Leu ($p > 0.10$). But, in STM, Lys decreased the expression around 70 pu ($p = 0.053$), whereas Leu increased it by 65% ($p = 0.070$). In liver, Lys alone substantially (more than 20×) increased ($p < 0.001$) the expression of CAT-1 mRNA; Leu alone ($p = 0.847$) or combined with Lys ($p = 0.458$) had no effect.

Discussion

The intestinal absorption and muscle cell uptake of CAAs is performed mainly by the heterodimeric unit

Treatment	Control	Lys	Leu	Lys × Leu			
L-Leucine	0.0	0.0	1.5	1.5	Basal vs.: (p)		
L-Lysine	0.0	3.5	0.0	3.5	Leu	Lys	Lys+Leu
$b^{0,+}$							
Jejunum	1.00	1.35	0.24	0.07	0.063	0.366	0.028
LDM	1.00	3.00	1.53	1.22	0.250	0.001	0.625
STM	1.00	0.56	1.01	0.53	0.986	0.067	0.050
Liver	1.00	0.68	3.02	0.35	0.110	0.786	0.585
CAT-1							
Jejunum	1.00	1.79	1.29	2.1	0.722	0.339	0.189
LDM	1.00	1.99	0.99	1.56	0.986	0.137	0.388
STM	1.00	0.31	1.65	1.15	0.070	0.053	0.657
Liver	1.00	20.70	0.39	3.37	0.847	0.001	0.458

Table 6 Relative expression of mRNA for $b^{0,+}$ and CAT-1 in jejunum, the *Longissimus dorsi* (LDM) and the *Semitendinosus* (STM) muscles, and liver of growing pigs in response to the individual or combined supplementation of L-Lys and Leu, as compared to the basal diet

rBAT/$b^{0,+}$AT (Broer, 2008) and the CAT-1 protein (Vekony et al., 2001) respectively, both are sodium-independent. According to Palacin et al. (2001), $b^{0,+}$ is the unit that catalyses transmembrane AA exchange; the $b^{0,+}$ system exchanges dibasic for neutral AAs. Broer (2008) indicates that the absorption of Lys by intestinal epithelial cells is strongly stimulated by Leu; this $b^{0,+}$ mediated process is coupled with the efflux of neutral AAs. Lys is the first limiting AA in the majority of feed ingredients for growing pigs, but Leu is abundantly supplied by protein-rich feed ingredients. The excess of Leu can be eliminated by reducing the protein level in the diet, and the Lys deficiency provoked by this reduction can be overcome with the supplementation of free L-Lys. Only a very few reports regarding the effect of dietary AA levels on the expression of cationic AA transporters in animals have been published.

In this study, the supplementation of L-Leu to the diet substantially reduced the expression of $b^{0,+}$ in jejunum; on average, pigs fed the L-Leu supplemented diets expressed less than one-sixth the $b^{0,+}$ gene as compared with those fed the diet with no supplemental L-Leu. Similar response was reported by Liao et al. (2009), who found that ruminal infusion of hydrolysed starch in steers, which increased the microbial protein and CAA supply, reduced the contents of the CAA-T systems y^+ and y^+L. These authors indicated that the down-regulation of CAA-T expression might reduce the absorption of unnecessary amounts of CAAs, thereby avoiding the energy cost and toxicity of metabolizing excess cationic AAs. This may partially explain the reduction in the expression of the $b^{0,+}$ transporter observed in this study. The adaptive regulation theory (Hatzoglou et al., 2004) implies that sufficient supply of AAs reduces the activity of the AA transporter (adaptive repression), but deficient AA intake increases the activity of the transporter (adaptive derepression). The response observed in this study suggests that supplemental L-Leu may decrease the activity of $b^{0,+}$ in jejunum.

The expression of $b^{0,+}$ in non-epithelial cells (muscle and liver) of this study varied depending on the tissue and type of muscle studied. In liver, the expression was reduced by supplemental L-Lys. In STM, Lys also reduced the expression of the transporter; this muscle contains fibres that are faster and more contractile than LDM and consume higher amounts of energy (Hemmings et al., 2009). Thus, the response in liver and STM to supplemental L-Lys can be related also to the adaptive regulation theory (Hatzoglou et al., 2004). In addition, it is generally accepted that absorbed AAs are transported via portal system to the liver, which distributes them to the whole body (van de Poll et al., 2007) and deaminates surplus AAs. Thus, it is possible that the AA profile in blood after leaving the liver is different from that in blood of the portal system right after being absorbed. Therefore, gene expression and activity of CAA-T could be expected to be different among the different muscles and tissues of the body.

Cationic AAs are also transported by the Na-independent CAT family proteins (Broer, 2008). CAT-1 possesses high affinity for Arg, Lys and ornithine (Hatzoglou et al., 2004). CAT-1 is ubiquitously expressed and its expression appears to be confined to the basolateral membrane (Vekony et al., 2001) and the non-epithelial cells (Broer, 2008). Hatzoglou et al. (2004) indicate that CAT-1 expression is regulated by growth factors and nutrients including AAs. Similarly, Fernandez et al. (2001) reported that CAT-1 expression in tissue cultures increased under condition of AA deficiency, through activation of the GCN2/eIF2α program (Majumder et al., 2009). In this study, Lys was not deficient and the highest

level was around 35% above the requirement for growing pigs, which may explain the lack of effect of these AAs on the expression of CAT-1 in jejunum and LDM. However, in STM, supplemental L-Lys reduced whereas L-Leu increased the expression of the transporter. Based on the findings by Fernandez et al. (2002), the reduction caused by supplementing L-Lys above the required level may indicate a high intracellular availability of Lys. Although, there is no clear explanation for the increase in the expression of CAT-1 observed with the high Leu levels, it is suggested that the highly dynamic interorgan AA exchange may be involved. Closs et al. (2006) indicated that CAT may deplete cells from CAA under certain circumstances because they also mediate their efflux. The most striking result was the substantial increase in the expression of CAT-1 in liver because of the supplementation of L-Lys. This effect is suggested as a mechanism of the body to more effectively take up and distribute the AAs to other tissues because liver functions as distributor of the absorbed AA (van de Poll et al., 2007). Therefore, the liver is expected to have a great capacity to absorb AAs prior to their distribution. In this regard, it is reasonable to speculate that the liver needs abundant and highly active AA transporters.

The expression of $b^{0,+}$ is higher in epithelial cells (Palacin et al., 2001), whereas CAT-1 is mostly expressed in non-epithelial cells (Vekony et al., 2001). However, there are no available reports showing the effect of dietary levels of AA on the expression of cationic AA transporters in tissues other than intestinal epithelium in mammals. In this study, $b^{0,+}$ was expressed in the small intestine, two muscles (LDM and STM) and liver, but the higher expression values were found in jejunum and LDM. In contrast, the expression of CAT-1 in jejunum was very low as compared to that observed in LDM and liver. These are consistent with the results found by Palacin et al. (2001) and Vekony et al. (2001). It appears that the uptake and catabolism of AAs by liver modifies the concentration of some AAs in systemic blood after leaving the liver. This could partially explain the differences in the expression of the AA transporter between intestinal and muscle cells. However, it is necessary to study in more detail the relationship between the expression and activity of AA transporters in the intestine and the AA concentration in portal blood of pigs fed diets with different levels of AAs. It is also important to determine whether the AA concentration in portal blood affects the activity of liver enzymes responsible for the catabolism of excess AAs recently absorbed and whether, as a result, the systemic AA concentration is modified.

In general, excess dietary levels of Leu reduces the expression of $b^{0,+}$ in jejunum, but had no effect on liver and STM. In addition, excess Leu levels in the diet do not affect the expression of CAT-1 in jejunum, LDM and liver, but reduce it in STM. On the other hand, excess dietary levels of Lys do not affect the expression of $b^{0,+}$ in jejunum, but reduce it in STM and liver. As for CAT-1, Lys excess did not affect its expression in jejunum and LDM, reduced it in STM, but increased it in liver. In conclusion, excess levels of Leu and Lys affect differently the expression of the cationic AA transporters $b^{0,+}$ and CAT-1 in growing pigs.

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